

Complexation between aluminum ion and glycolic acid under acidic condition

Mayumi Etou (✉ etou-mayumi@oita-u.ac.jp)

Oita University

Toshifumi Taketatsu

Kyushu University

Yoshihiro Okaue

Kyushu University

Takanori Inoue

Oita University

Takushi Yokoyama

Kyushu University

Research Article

Keywords: Aluminum ion, Complexation, Glycolic acid, Acidic condition

Posted Date: July 5th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1786861/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Journal of Solution Chemistry on August 8th, 2023. See the published version at <https://doi.org/10.1007/s10953-023-01319-7>.

Abstract

The complexation between Al^{3+} ($[\text{Al}(\text{H}_2\text{O})_6]^{3+}$) and glycolic acid (GA, $\text{C}_2\text{H}_4\text{O}_3$) which has a carboxyl group and a hydroxyl group in a molecule was investigated under acidic condition using ^{27}Al NMR, ^{13}C NMR and ESI-MS techniques. The five peaks including a peak due to Al^{3+} were observed in ^{27}Al NMR spectra for the mixed solution of Al^{3+} and GA, suggesting the existence of at least four Al-GA complexes. The results of NMR and ESI-MS measurements revealed that GA and Al^{3+} can form one monodentate complex (AlGA^{2+}) and three bidentate complexes (AlGA^+ , AlGA_2^- , and AlGA_3^{3-}) complexes. From the deconvolution of ^{27}Al NMR spectra and pKa value of GA, the conditional formation constants ($\log K$) of each complex (GA/Al molar ratio of 25 in mixed solution) can be determined to be 0.94 (AlGA^{2+}), -0.96 (AlGA^+), -0.77 (AlGA_2^-) and -2.21 (AlGA_3^{3-}), respectively. In addition, the overall formation constant of three bidentate complex at pH 3 was also calculated to be -1.65.

1. Introduction

Aluminium (Al) is the most abundant metal ion (approximately 7%) in earth's crust. Under ordinary conditions, Al is fixed as Al^{3+} in natural solids such as rocks and soils. However, in acidified environments, Al is released as hexaaqua aluminum ion ($[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, Al^{3+}) and its hydrolytic species (AlOH^2 , $\text{Al}(\text{OH})$, and the tridecameric Al polymer (the Keggin-type Al_{13} polycation)) into the soil solution due to decomposition of soil minerals by attack of H^+ . As a result, it can easily mobilize in aquatic environments [1]. In addition, these dissolved Al^{3+} species exhibit significant toxicity towards plants and animals. For example, several studies have reported that even micromolar concentrations of Al^{3+} can inhibit plant growth and production [2–7], and this toxicity disappeared upon the formation of organic and inorganic Al^{3+} complexes [2, 4, 7–11].

Many Al-tolerant plants release organic acids, especially carboxylic acids, that can complex Al^{3+} , consequently reducing the toxicity of Al^{3+} [2–7]. Therefore, the interactions between Al^{3+} and such simple organic acids have been intensively investigated [12–18]. In addition, humic substances (HS) which are abundant natural organic compounds are also able to form Al-HS complexes in the environment. The complexation between Al^{3+} and HS plays an important role in reducing the toxicity and mobility of Al^{3+} and in natural waters [19–22]. The structure of HS is very complicated and the main functional groups are carboxylic group and hydroxyl group [23–27]. Although, the complexation between Al^{3+} and HS is very important, it is difficult to determine the functional groups in HS that are behind this complexation [28]. Therefore, a model compound with the local structure of HS has been used to study its interaction with Al^{3+} [28–30]. In a previous study, we investigated the interaction between Al^{3+} and synthesized 2, 3-dihydroxyterephtharic acid (DHTPA), an aromatic compound bearing two carboxylic and two hydroxyl groups, as a model compound with the functional groups of fluvic acids which is a soluble HS in water both under acid and alkaline conditions [28]. The average conditional stability constant of the Al-DHTPA complex was successfully calculated by nuclear magnetic resonance (NMR) and potentiometric titration.

The use of such model compound is useful to determine the interaction between Al^{3+} and local structure of fulvic acid.

Since the DHTPA is an aromatic compound with two carboxyl groups and two hydroxyl groups in the molecule, in this work, glycolic acid (hydroxyacetic acid, $\text{C}_2\text{H}_4\text{O}_3$) (GA) which is an aliphatic compound with one carboxyl group and one hydroxyl group was selected to examine the interaction between Al^{3+} and a local structure of fulvic acid. As the GA is a natural organic compound found especially in sugar-related plants and has one carboxyl group and one hydroxyl group in the molecule, it is reasonable to use GA as the model compound of local structure of HS. In addition, the GA is the smallest molecule having a carboxylic group and a hydroxyl group within a molecule. The interaction with metal ions is interesting both on complex chemistry and on environmental chemistry. In this study, the complexation between Al^{3+} and GA was investigated using ^{27}Al and ^{13}C NMR and ESI-MS techniques. All experiments were conducted at pH 3, which is a limiting pH of acidified soil so as to avoid the hydrolysis of Al^{3+} .

2. Experimental

2.1 Reagents and solutions

Aluminum (Al) stock solution ($0.1 \text{ mol}\cdot\text{dm}^{-3}$) were prepared by dissolving crystalline aluminum nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$, Wako Pure Chemicals) in 0.1 mol dm^{-3} nitric acid. The Al concentration was determined by EDTA titration. Glycolic acid (GA) (pK_a : 3.83) solution with the desired concentrations was prepared by dissolving the GA (Tokyo Chemical Industry Co., Ltd.) in water. All sample solutions were prepared with ultrapure water (Milli-Q SP system, Millipore). Sample solutions were prepared by mixing the Al stock solution with the GA stock solution in the desired molar ratio (GA/Al ratio). Mixed solutions of Al and GA with desired GA/Al molar ratio were adjusted to pH 3 by dropping a sodium hydroxide solution and nitric acid. ^{27}Al and ^{13}C NMR spectra and ESI-MS spectra of the mixed solutions were measured.

2.2 Measurement of ^{27}Al and ^{13}C NMR spectra

The ^{27}Al NMR spectra were recorded on a JEOL ECA-600 spectrometer operating at 156.39 MHz. (repetition period: 0.37 s, acquisition time: 0.262 s). Each sample solution was placed in a 5 mm quartz tube. A Sodium aluminate dissolved in D_2O enclosed in quartz tube with a diameter of 2 mm was placed into 5 mm quartz tube as an external standard. Chemical shift were referenced to the signal of $0.02 \text{ mol}\cdot\text{dm}^{-3}$ $[\text{Al}(\text{OD})_4]^-$ solution (80 ppm from $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$). The ^{13}C NMR spectra were also recorded on a JEOL ECA-600 spectrometer operating at 150.91 MHz (repetition time: 1.19 s, acquisition time: 0.693 s). Chemical shift was referenced to the signal of TMS.

2.3 Measurement of ESI- MS spectra

ESI-MS measurement of mixed solution of Al^{3+} and GA was performed using a Waters ESI-MS spectrometer, Quattromicro API in the positive ionization mode. The solutions were introduced into the

spectrometer at a flow rate of 2 uL/min. The operation conditions were as follows: capillary voltage 3.0 kV, sample cone voltage 50V, RF lens 0 V, source temperature 120 °C, desolvation temperature 150 °C, cone gas flow rate 50L/h and desolvation gas flow rate 600 L/h.

3. Results And Discussion

3.1 Interaction between Al³⁺ and GA in solution at pH 3

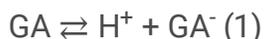
Figure 1 shows the ²⁷Al NMR spectra of [Al(H₂O)₆]³⁺ and glycolic acid (GA) mixed solutions at various GA/Al molar ratio at pH 3. The total Al concentration in sample solutions was 0.02 mol·dm⁻³. The sharp peak at 0 ppm (* in Fig. 1) is due to [Al(H₂O)₆]³⁺, and at least four new broad peaks were observed by the peak separation (shown in Fig. 2). The chemical shift values of the peaks from the [Al(H₂O)₆]³⁺ were 0.28, 9.8, 17.8, and 26.2 ppm, respectively. Consequently, these new peaks are attributed to the Al-GA complexes. Based on the chemical shift values, the Al³⁺ in the Al-GA complex is 6-coordinated. From the peak intensities of the ²⁷Al NMR spectra, the distribution of each Al³⁺ as a function of the GA/Al molar ratio was calculated (Fig. 3). When the GA/Al ratio was < 5, the dominant form of Al was free [Al(H₂O)₆]³⁺. On the other hands, the GA/Al ratio was ≥ 5, the Al-GA complexes became dominant, and at a molar ratio of 25, almost all Al³⁺ species were under the form of the Al-GA complex. In the new peaks present due to the Al-GA complexes, the first peak at 0.28 ppm has a slight downfield shift from the peak at 0 ppm due to [Al(H₂O)₆]³⁺. Etou *et al.* reported the interaction between Al³⁺ and acrylic acid (AA) at pH 3 and suggested the formation of a 1 : 1 monodentate Al-AA complex because of the slight downfield shifts (1 ppm) [29]. In this study, the peak at 0.28 ppm was assigned to the 1 : 1 monodentate complex. From the pKa value, the binding site of GA in the monodentate complex may be a carboxyl group. The difference in the chemical shift of the three other peaks was similar, and the peak intensity increased with increasing GA/Al ratio, thus suggesting the formation of successive complexes.

Both functional groups of GA (the carboxyl group and hydroxyl group) can bind to Al³⁺ by a ligand exchange reaction. At a GA/Al molar ratio of 2, the dominant Al-GA complex was observed at 9.8 ppm in the ²⁷Al NMR spectrum, while other complexes were not abundant (Fig. 3). To determine the coordination site of GA in the Al-GA complex, ¹³C NMR spectra were recorded for a GA solution and the mixed solution of GA/Al of 2 (Fig. 4). The peaks at 179.7 and 62.5 ppm were attributed to carbon atoms in the carboxyl group and hydroxymethyl group in free GA (Fig. 4 (a), ▼). In contrast, in the mixed solution, the peaks corresponding to free GA were not observed, and new peaks at 180.6 and 64.1 ppm were observed (Fig. 4(b), ▽) which can be assigned to the carboxyl and hydroxymethyl groups in the Al-GA complex. From the ¹³C NMR spectra, the binding sites of GA with Al³⁺ were both carboxylic and hydroxymethyl groups. In addition, the ESI-MS spectrum of the same sample is shown in Fig. 5. The peak at m/z = 137.03 (●) is attributed to [Al(OCH₂COO)(H₂O)₂]⁺. From the results of ¹³C NMR and ESI-MS measurements, the peak at 9.8 ppm in ²⁷Al NMR spectra of the mixed solution with GA/Al molar ratio of 2

could be identified as a 1 : 1 AlGA⁺ bidentate complex. It was also shown that Al³⁺ formed a bidentate complex with carboxyl and a hydroxylmethyl groups. Since the additivity low of chemical shift was established for the three other peaks of Al-GA complexes (9.8, 17.8, and 26.2 ppm), it was considered that 1 : 1 to 1 : 3 chelate complexes were successively formed. These results indicate that GA can form monodentate and bidentate complexes with Al³⁺. The structural formulae of the suggested Al-GA complexes are shown in Fig. 6.

3.2 Conditional formation constants of Al-GA complexes

The results revealed that GA formed multiple complexes with Al³⁺ at pH 3. Successively, the conditional formation constant (log *K*) of each complex and the overall formation constant of three bidentate were determined from the ²⁷Al NMR results. Considering the pKa value of GA (3.83) (1), the reactions with Al³⁺ were considered as (3), (5), (7), and (9). From the pKa value, it was found that approximately 13% of carboxylic groups dissociated at pH 3. The hydration of Al³⁺ was omitted for simplicity. Using the result of peak separation from the ²⁷Al NMR spectra and the total Al³⁺ and GA concentrations, which were determined in the preparation of the solutions, the concentration of each complex was determined. Here, *K_a*, *K_{1m}*, *K_{1b}*, *K_{2b}*, and *K_{3b}* indicate the dissociation constant of GA, and the formation constants of 1:1 monodentate, 1:1 bidentate, 1:2 bidentate, and 1:3 bidentate Al-GA complexes, respectively.



$$K_a = [\text{H}^+][\text{GA}^-]/[\text{GA}] = 1.50 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3} \quad (2)$$

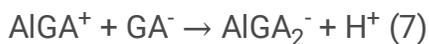


$$K_{1m} = [\text{AlGA}^{2+}]/[\text{Al}^{3+}][\text{GA}^-] \quad (4)$$



In the formation of the bidentate complex, H⁺ was released from an OH group.

$$K_{1b} = [\text{AlGA}^+][\text{H}^+]/[\text{Al}^{3+}][\text{GA}^-] \quad (6)$$



$$K_{2b} = [\text{AlGA}_2^-][\text{H}^+]/[\text{AlGA}^+][\text{GA}^-] \quad (8)$$



$$K_{3b} = [\text{AlGA}_3^{3-}][\text{H}^+]/[\text{AlGA}_2^-][\text{GA}^-] \quad (10)$$

From the mass balance equation of Al³⁺ and GA, the total Al and GA concentrations were calculated as follows. [H⁺] was constant at 10⁻³ mol·dm⁻³ (pH 3).

$$[\text{Al}]_{\text{total}} = [\text{Al}^{3+}] + [\text{AlGA}^{2+}] + [\text{AlGA}^+] + [\text{AlGA}_2^-] + [\text{AlGA}_3^{3-}] \quad (11)$$

If $[\text{Al}]_{\text{total}}$ is known, $[\text{Al}^{3+}]$, $[\text{AlGA}^{2+}]$, $[\text{AlGA}^+]$, $[\text{AlGA}_2^-]$ and $[\text{AlGA}_3^{3-}]$ can be calculated from the peak separation of the ^{27}Al NMR spectra.

$$[\text{GA}]_{\text{total}} = [\text{GA}] + [\text{GA}^-] + [\text{AlGA}^{2+}] + [\text{AlGA}^+] + 2[\text{AlGA}_2^-] + 3[\text{AlGA}_3^{3-}] \quad (12)$$

$$[\text{GA}^-] = ([\text{GA}]_{\text{total}} - [\text{AlGA}^{2+}] - [\text{AlGA}^+] - 2[\text{AlGA}_2^-] - 3[\text{AlGA}_3^{3-}]) / (1 + [\text{H}^+]/K_a) \quad (13)$$

As $[\text{GA}]_{\text{total}}$, $[\text{AlGA}^{2+}]$, $[\text{AlGA}^+]$, $[\text{AlGA}_2^-]$, $[\text{AlGA}_3^{3-}]$, $[\text{H}^+]$ and K_a are known, $[\text{GA}^-]$ can be easily calculated. Consequently, the formation constant of equations (4), (6), (8) and (10) can be calculated.

The log K values of the complexes at pH 3 were present in Table 1. In addition, from these results, the overall formation constant of three bidentate complexes at a GA/Al molar ratio of 25 was calculated. The overall reaction and the overall formation constant ($\log\beta$) of bidentate complexes are represented as (14) and (15). The $\log\beta$ was calculated to be -1.65.



$$\beta = [\text{Al}(\text{GA})_3^{3-}][\text{H}^+]^3 / [\text{Al}^{3+}][\text{GA}^-]^3 \quad (15)$$

Table 1
Conditional formation constants of Al-GA complexes.

GA/Al molar ratio in mixed solution	2	5	10	25
$\log K_{1m}$	0.99	1.07	1.32	0.94
$\log K_{1b}$	-1.12	-1.00	-0.90	-0.96
$\log K_{2b}$	-1.07	-0.91	-1.02	-0.77
$\log K_{3b}$	-	-	-3.01	-2.21

This study revealed that stable GA-Al complexes can be formed at pH 3. From the above results, when carboxyl and hydroxyl groups are present in the vicinity of a natural insoluble organic macromolecule which have a complicated structure, such as humic acid, they can act as a reaction site for immobilizing Al^{3+} . In addition, in terms of the interaction between Al^{3+} and simple carboxylic acid, several studies have been conducted on the complexation between Al^{3+} and simple dicarboxylic acids. However, this study revealed that even a simple organic acid bearing only one carboxylic and one hydroxyl group, such as GA, can form a chelate structure and stable complexes in the environment. These results strongly suggest that fulvic acid and simple organic compounds in the soil can effectively decrease in the toxicity of Al^{3+} dissolved in an acidic environment.

4. Conclusion And Implication In Environmental Chemistry

The complexation of Al^{3+} and GA was investigated at pH 3. GA has a one carboxyl and one hydroxymethyl groups in the structure and has a possibility of the formation of chelate structure with Al^{3+} . The NMR and ESI-MS results revealed that GA can form various Al-GA complexes, including a monodentate complex and three bidentate complexes (AlGA^+ , AlGA_2^- , and AlGA_3^{3-}) at pH 3. The log K of each complex were determined from ^{27}Al NMR measurement. The log K value of each complex was calculated and the overall formation constant of three bidentate complex was also determined (log β : -1.65). The chelating effect of the carboxylic and hydroxymethyl groups can render Al^{3+} immobile even in acidic conditions and consequently decrease its toxicity in hydrosphere.

Declarations

Acknowledgement

The measurements of ^{27}Al and ^{13}C NMR and ESI-MS were made at the Center of Advanced Instrument Analysis, Kyushu University.

Conflict of interest

The authors hereby declare no existing financial interests concerning these research studies.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mayumi Etou and Toshifumi Taketatsu. The first draft of the manuscript was written by Mayumi Etou and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Vance, G.F., Stevebson, F.J., Sikora, F.J., Sposito, G. (eds.): The Environmental Chemistry of Aluminum. Lewis Publishers, New York (1996)
2. Ma, J.F., Ryan, P.R., Delhaize, E.: Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Sci.* **6**, 273–278 (2001). [https://doi.org/10.1016/S1360-1385\(01\)01961-6](https://doi.org/10.1016/S1360-1385(01)01961-6)
3. Mhatre, S.N., Iyer, R.K., Moorthy, P.N.: Characterization of Aluminium Complexes in Tea Extract. *Magn. Reson. Chem.* **31**, 169–175 (1993). <https://doi.org/10.1002/mrc.1260310203>
4. Zheng, S.J., Ma, J.F., Matsumoto, H.: High Aluminum Resistance in Buckwheat. *Plant. Physiol.* **117**, 745–751 (1998). <https://doi.org/10.1104/pp.117.3.745>
5. Morita, A., Horie, H., Fujii, Y., Takatsu, S., Watanabe, N., Yagi, A., Yokota, H.: Chemical forms of aluminum in xylem sap of tea plant (*Camellia sinensis* L.). *Phytochemistry.* **65**, 2775–2780 (2004).

<https://doi.org/10.1016/j.phytochem.2004.08.043>

6. Huang, D., Gong, Z., Chen, X., Wang, H., Tan, R., Mao, Y.: Transcriptomic responses to aluminum stress in tea plant leaves. *Sci. Rep.* **11**, 5800 (2021). <https://doi.org/10.1038/s41598-021-85393-1>
7. Pellet, D.M., Grunes, D.L., Kochian, L.V.: Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). *Planta.* **196**, 788–795 (1995). <https://doi.org/10.1007/BF01106775>
8. Delhaize, E., Ryan, P.R.: Aluminum Toxicity and Tolerance in Plants. *Plant. Physiol.* **107**, 315–321 (1995). <https://doi.org/10.1104/pp.107.2.315>
9. Xiao, Z., Liang, Y.: Silicon prevents aluminum from entering root tip by promoting formation of root border cells in rice. *Plant. Phys. Biochem.* **175**, 12–22 (2022). <https://doi.org/10.1016/j.plaphy.2022.02.003>
10. Hodson, M., Evans, D.E.: Aluminium-silicon interactions in higher plants: an update. *J. Exp. Botany.* **71**(21), 6719–6729 (2020). <https://doi.org/10.1093/jxb/eraa024>
11. Corbillon, M.S., Olazabal, M.A., Madariaga, J.M.: Potentiometric Study of Aluminium-Fluoride Complexation Equilibria and Definition of the Thermodynamic Model. *J Solut. Chem.* **37**, 567–579 (2008). <https://doi.org/10.1007/s10953-008-9257-3>
12. Cardiano, P., Giacobello, F., Giuffrè, O., Sammartano, S.: Thermodynamics of Al^{3+} -thiocarboxylate interaction in aqueous solution. *J. Mol. Liquid.* **222**, 614–621 (2016). <http://dx.doi.org/10.1016/j.molliq.2016.07.077>
13. Cardiano, P., Giacobello, F., Giuffrè, O., Sammartano, S.: Thermodynamics and spectroscopic study on Al^{3+} -polycarboxylate interaction in aqueous solution. *J. Mol. Liquid.* **232**, 45–54 (2017). <https://doi.org/10.1016/j.molliq.2017.02.047>
14. Torre, G.D., Mujika, J.I., Formoso, E., Matito, E., Ramos, M.J., Lopez, X.: Tuning the affinity of catechols and salicylic acids towards Al(III) : characterization of Al – chelator interactions. *Dalton Trans.* **47**, 9592–9607 (2018). <https://doi.org/10.1039/C8DT01341A>
15. Etou, M., Kurisaki, T., Okaue, Y., Wakita, H., Yokoyama, T.: ^{13}C and ^{27}Al NMR Study of Complexation between Aluminium Ion and Simple Dicarboxylic Acids under an Acidic Condition: New Peak Assignments of ^{27}Al NMR Spectra of Mixed Solutions of Al^{3+} and Simple Dicarboxylic Acids. *Anal. Sci.* **29**, 843–844 (2013). <https://doi.org/10.2116/analsci.29.843>
16. Yokoyama, T., Abe, H., Kurisaki, T., Wakita, H.: ^{13}C and ^{27}Al NMR and Potentiometric Study on the Interaction between Aluminium Ions and Quinolic Acids in Acidic Aqueous Solutions. *Anal. Sci.* **15**, 969–972 (1999). <https://doi.org/10.2116/analsci.15.969>
17. Rubini, P., Lakatos, A., Champmartin, D., Kiss, T.: Speciation and structural aspects of interactions of Al(III) with small biomolecules. *Coord. Chem. Rev.* **228**, 137–152 (2002). [https://doi.org/10.1016/S0010-8545\(01\)00467-2](https://doi.org/10.1016/S0010-8545(01)00467-2)
18. Boily, J.F., Qafoku, O., Felmy, A.R.: A Potentiometric, Spectrophotometric and Pitzer Ion-Interaction Study of Reaction Equilibria in the Aqueous H^+ - Al^{3+} , H^+ -Oxalate and H^+ - Al^{3+} -Oxalate Systems up to 5

- $\text{mol}^{1/2}\text{dm}^{-3}$ NaCl. *J Solut. Chem.* **36**, 1727–1743 (2007). <https://doi.org/10.1007/s10953-007-9203-9>
19. Fakhraei, H., Driscoll, C.T.: Proton and Aluminum Binding Properties of Organic Acids in Surface Waters of the Northeastern U. S. *Environ. Sci. Technol.* **49**, 2939–2947 (2015). <https://doi.org/10.1021/es504024u>
 20. Takahashi, T., Nanzyo, M., Hiradate, S.: Aluminum status of synthetic Al-humic substance complexes and their influence on plant root growth. *Soil. Sci. Plant. Nutr.* **53**, 115–124 (2007). <https://doi.org/10.1111/j.1747-0765.2007.00114.x>
 21. Burba, L.J.B.J.: Evaluation of conditional stability constants of dissolved aluminum/humic substance complexes by means of ^{27}Al nuclear magnetic resonance. *Fresenius J. Anal. Chem.* **351**, 83–87 (1995). <https://doi.org/10.1007/BF00324295>
 22. Pourpoint, F., Templier, J., Anquetil, C., Vezin, H., Trébosc, J., Trivelli, X., Chabaux, F., Pokrovsky, O.S., Prokushkin, A.S., Amoureux, J.P., Lafon, O., Derenne, S.: Probing the aluminum complexation by Siberian riverine organic matter using solid-state DNP-NMR. *Chem. Geol.* **452**, 1–8 (2017). <https://doi.org/10.1016/j.chemgeo.2017.02.004>
 23. Leenheer, J.A., Wershaw, R.L., Reddy, M.M.: Strong-Acid, Carboxyl-Group Structures in Fulvic Acid from the Suwannee River, Georgia. 1. Minor Structures. *Environ. Sci. Technol.* **29**, 393–398 (1995). <https://doi.org/10.1021/es00002a015>
 24. Leenheer, J.A., Wershaw, R.L., Reddy, M.M.: Strong-Acid, Carboxyl-Group Structures in Fulvic Acid from the Suwannee River, Georgia. 2. Major Structures. *Environ. Sci. Technol.* **29**, 399–405 (1995). <https://doi.org/10.1021/es00002a016>
 25. Atalay, Y.B., Carbonaro, R.F., Di Toro, D.M.: Distribution of Proton Dissociation Constants for Model Humic and Fulvic Acid Molecules. *Environ. Sci. Technol.* **43**, 3626–3631 (2009). <https://doi.org/10.1021/es803057r>
 26. Nimmagadda, R.D., AcRae, C.: Characterisation of the backbone structures of several fulvic acids using a novel selective chemical reduction method. *Org. Geochem.* **38**, 1061–1072 (2007). <https://doi.org/10.1016/j.orggeochem.2007.02.016>
 27. Baigorri, R., Fuentes, M., Conzález-Gaitaro, G., Marcía-Mina, J., Almendros, G., González-Vila, F.J.: Complementary Multianalytical Approach To Study the Distinctive Structural Features of the Main Humic Fractions in Solution: Gray Humic Acid, Brown Humic Acid, and Fulvic Acid. *J. Agric. Food Chem.* **57**, 3266–3272 (2009). <https://doi.org/10.1021/jf8035353>
 28. Kurisaki, T., Etou, M., Okaue, Y., Wakita, H., Yokoyama, T.: Acid-base behavior and Al^{3+} complex formation of synthesized 2,3-dihydroxyterephthalic acid (DHTPA) at pH 3 as a model compound of Inogashira fulvic acid (IFA). *Polyhedron.* **72**, 135–139 (2014). <https://doi.org/10.1016/j.poly.2014.02.006>
 29. Etou, M., Masaki, Y., Tsuji, Y., Saito, T., Bai, S., Nishida, I., Okaue, Y., Yokoyama, T.: Interaction between Al^{3+} and Acrylic Acid and Polyacrylic Acid in Acidic Aqueous Solution: A Model Experiment for the

Behavior of Al^{3+} in Acidified Soil Solution. Anal. Sci. **27**, 111–115 (2011).

<https://doi.org/10.2116/analsci.27.111>

30. Yokoyama, T., Abe, H., Kurisaki, T., Wakita, H.: ^{13}C and ^{27}Al NMR Study on the Interaction in Acidic Aqueous Solution between Aluminium Ion and Tiron, Salicylic Acid and Phthalic Acid: as Model Compounds with Functional Groups of Fulvic Acid. Anal. Sci. **13**, 425–428 (1997).

https://doi.org/10.2116/analsci.13.Supplement_425

Figures

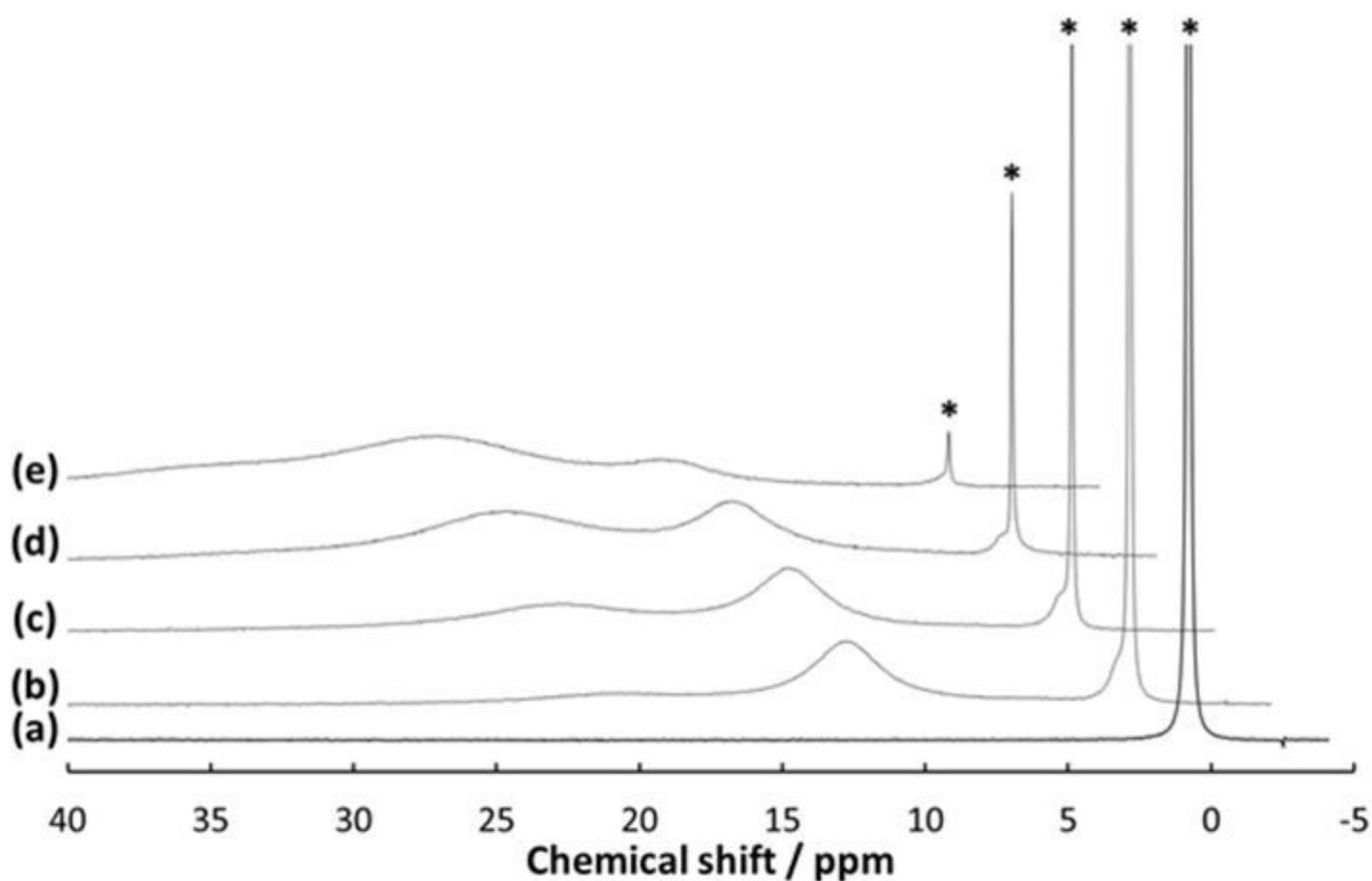


Figure 1

^{27}Al NMR spectra of the mixed solutions of Al^{3+} and GA at pH 3 with various GA/Al molar ratios. (a) $\text{Al}(\text{NO}_3)_3$ solution at pH 3. GA/Al = (b) 2, (c) 5, (d) 10, and (e) 25.

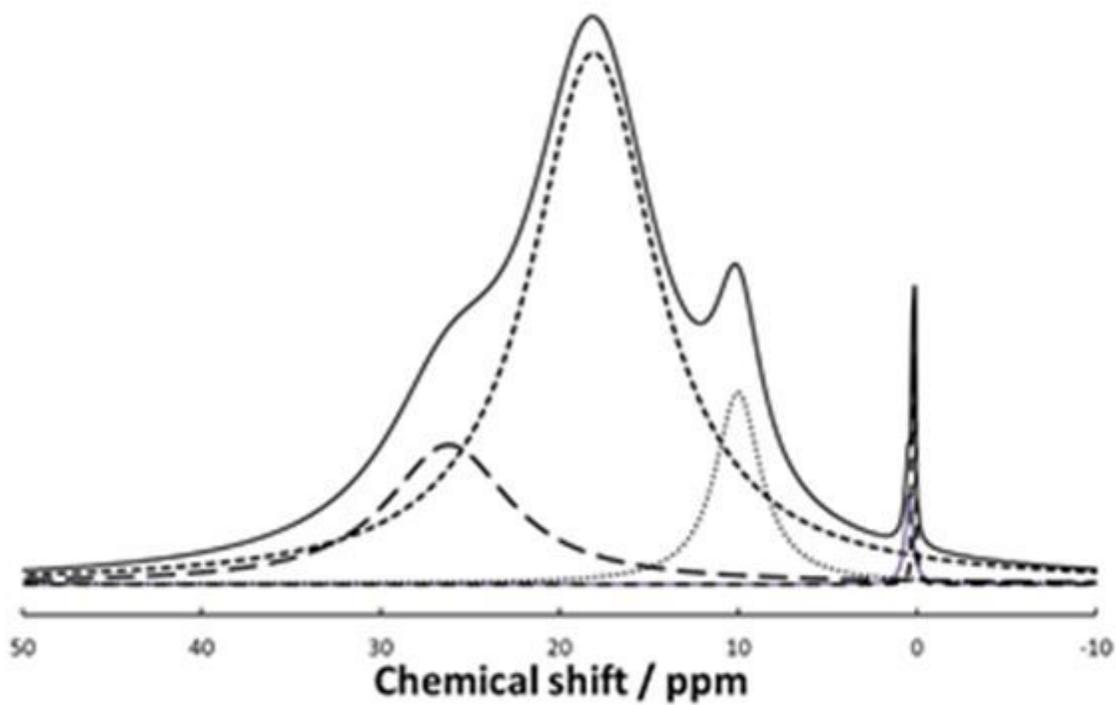


Figure 2

Peak separation of the ^{27}Al NMR spectrum of a mixed solution of Al^{3+} and GA (GA/Al = 25) at pH 3.

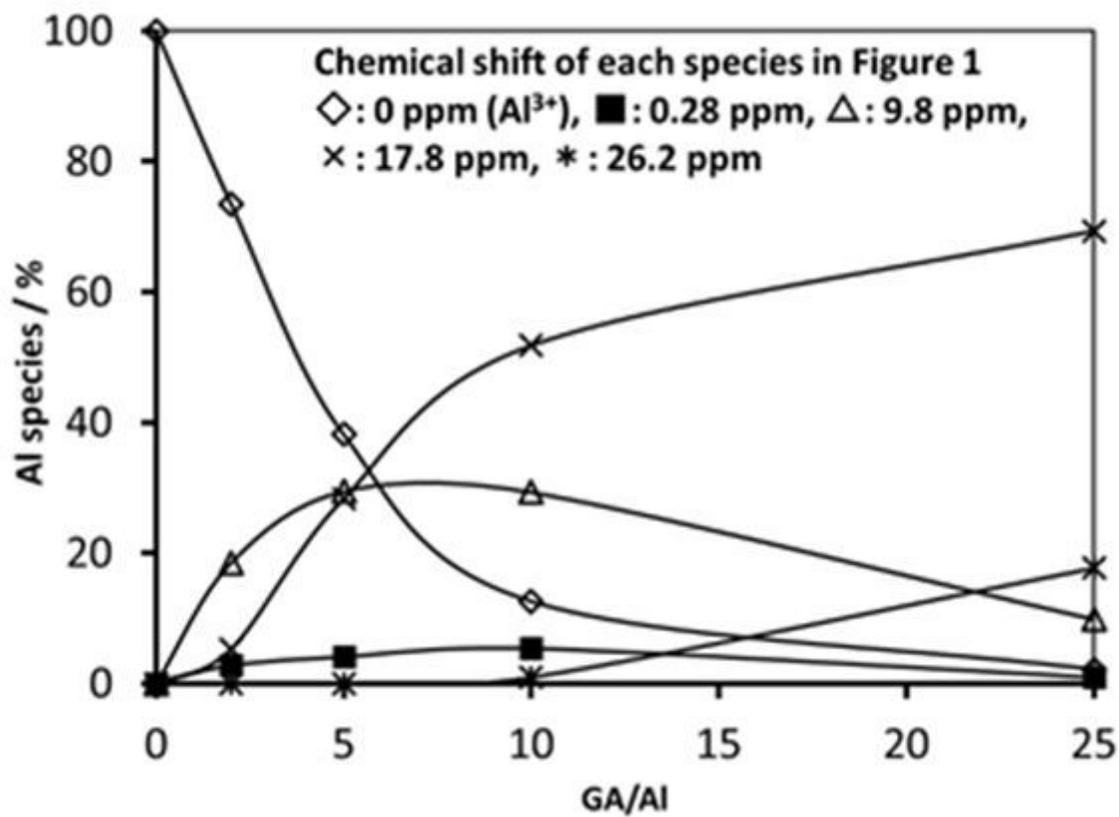


Figure 3

Distribution curves of Al species as functions of GA/Al molar ratios at pH 3.

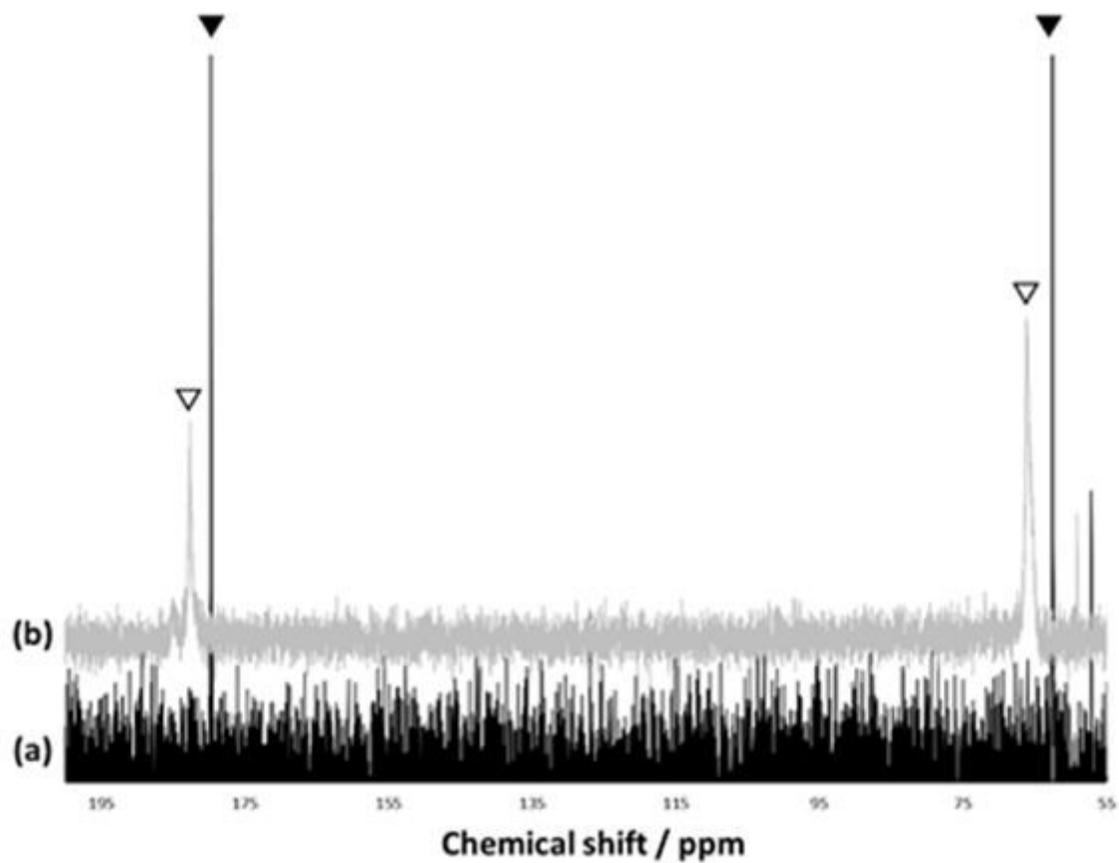


Figure 4

^{13}C NMR spectra of a mixed solution of Al^{3+} and GA at pH 3. (a) GA solution, (b) GA/Al = 2.

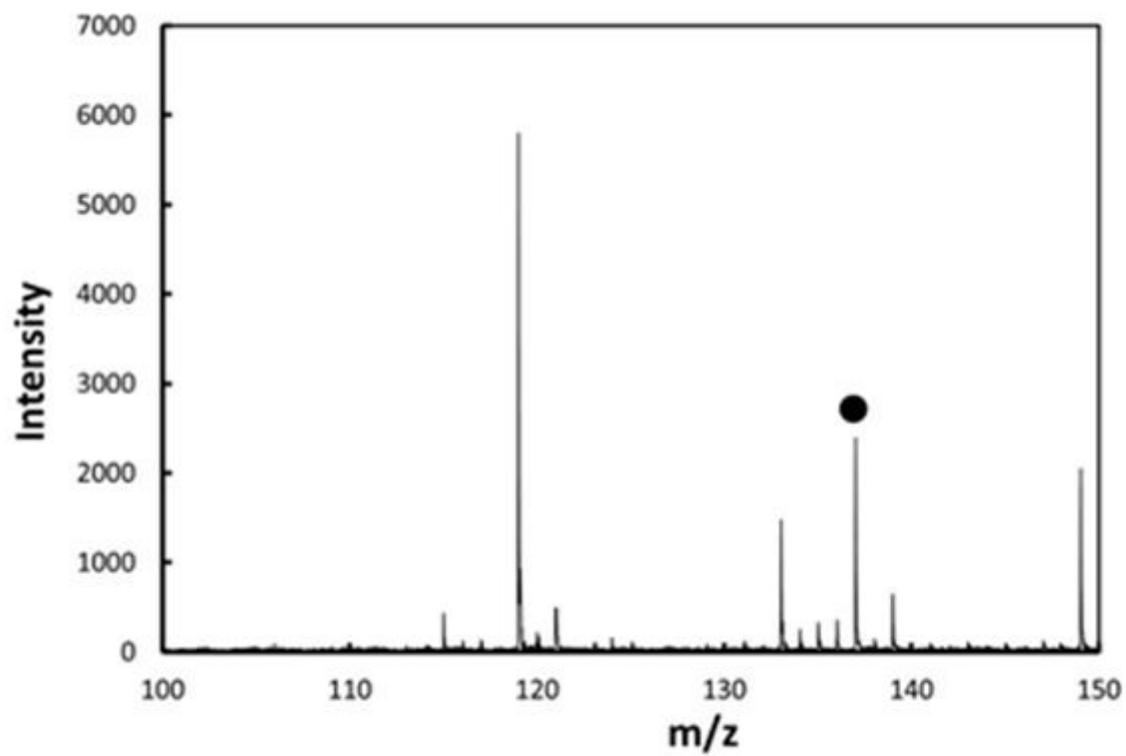
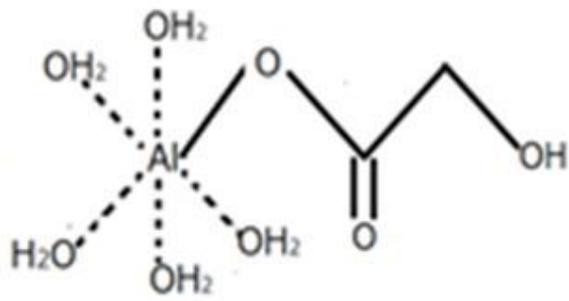
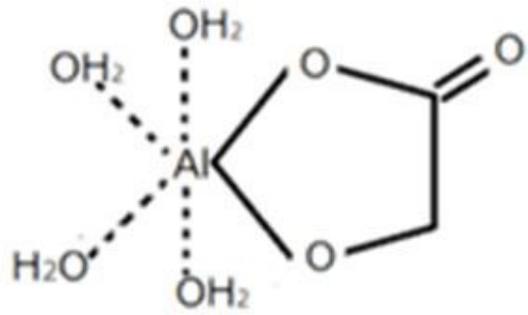


Figure 5

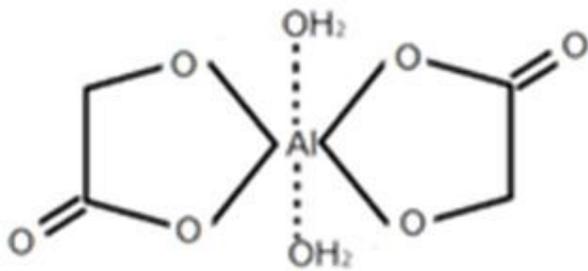
ESI-MS spectrum of a mixed solution of Al^{3+} and GA (GA/Al = 2) at pH 3.



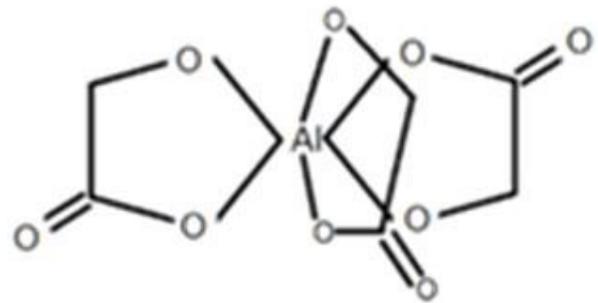
1 : 1 complex (monodentate)



1 : 1 complex (bidentate)



1 : 2 complex (bidentate)



1 : 3 complex (bidentate)

Figure 6

Proposed structures of Al-GA complexes.